of the Treg lineage because Treg cells derived from mice with a disabled Foxp3 gene express many, but not all, of the phenotypic and functional properties of normal Treg cells. Thus, the signal(s) responsible for the initiation of Treg cell development remains unknown, and it remains possible that one result of commitment to the Treg cell lineage is an increased resistance to negative selection in the thymus resulting in a population of Treg cells with a TCR repertoire similar to Tconv cells but with slightly higher affinity to self. The self-reactivity might be below the threshold detectable in a T cell hybridoma. Although the studies of Pacholczyk et al. (2007) indicate that the T cells that initiate wasting disease recognize nonself, it is premature to conclude that the Treg cells that protect from wasting disease also exclusively recognize nonself. Self-specific Treg cells might play a critical early role in preventing disease by reacting to enhanced amounts of self-peptide MHC class II and mediating bystander suppression. This model does not exclude the possibility that foreign antigen-specific Treg cells play a complementary role in protection.

Lastly, one must also consider the implications of these studies of TCR repertoire to vaccination. In contrast to the well-described in vitro anergic state of Foxp3$^+$ Treg cells, they proliferate and expand as efficiently as do Tconv cells after in vivo priming. Because the TCR repertoires of Treg and Tconv cells are certainly capable of recognizing any foreign or pathogen-derived antigen, it is likely that vaccination will result in proliferation of both populations. Because the marked proliferation of Treg cells in the tumor-bearing host can be secondary to their higher affinity for tumor (self-) antigens, tumor vaccines can result in further proliferation of the tumor-specific Treg cells. Manipulation of the balance between Treg and Tconv cells in response to vaccination remains a challenge for the future.

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Crosspresentation: Plasmacytoid Dendritic Cells Are in the Business

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Crosspriming and crosspresentation are performed by specialized subsets of dendritic cells. In this issue, Hoeffel et al. (2007) show that human plasmacytoid dendritic cells can crosspresent HIV-derived peptides conjugated to a lipopeptide or HIV-infected cells undergoing apoptosis.

"Exogenous antigens are loaded onto major histocompatibility complex (MHC) class II molecules, whereas endogenous antigens are loaded onto MHC class I molecules" is a golden rule with notable exceptions. Pioneering work showed that antigen-specific CD8$^+$ T cells expand in vivo when small amounts of exogenous antigen are delivered together with dead or dying cells, a phenomenon designated crosspriming or crosspresentation for memory responses. A few years later, several groups demonstrated that a small fraction of dedicated dendritic cells (DCs) within the DEC205$^+$CD8α^+ subset performs crosspriming (Bevan, 2006). Since then, the relevance of crosspriming in immune responses has been the subject of extensive investigation. Certainly, extending our knowledge of the key players and molecular mechanisms involved in crosspriming and crosspresentation might allow the experimental manipulation of this pathway for therapeutic intervention in cancer and autoimmunity. In this issue of *Immunity*, Hoeffel et al. (2007) show that human plasmacytoid DCs (pDCs) can crosspresent HIV-derived antigens.

Under steady-state conditions, crosspresentation provides tolerance

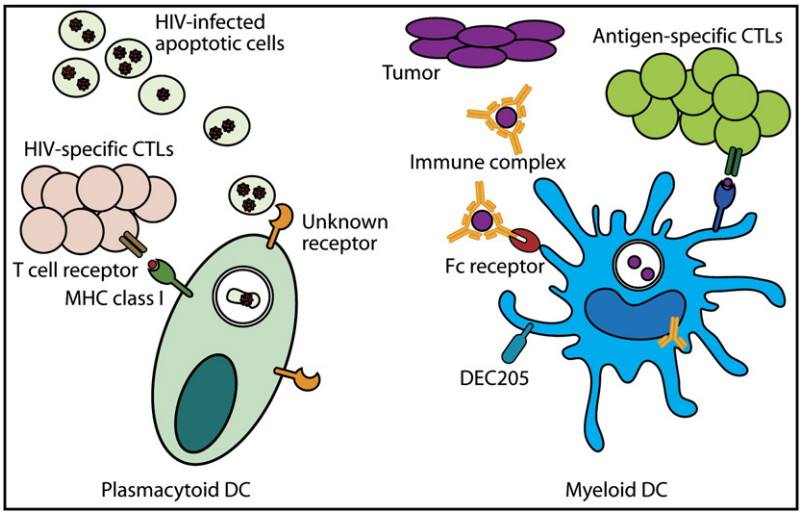

Figure 1. Functional Specialization of Human DC Subsets in Crosspresentation of Exogenous Antigens

Plasmacytoid DCs crosspresent HIV-infected cells undergoing apoptosis to HIV-specific CTLs. The nature and specificity of the receptor(s) mediating uptake and crosspresentation has yet to be established. Myeloid DCs crosspresent apoptotic tumor-transformed cells or tumor-derived antigens offered as immune complexes to antigen-specific CTLs. Immune complexes are taken up through Fc receptors. Myeloid DCs also efficiently crosspresent soluble antigens targeted to endocytic receptors, such as DEC205.

by inducing peripheral deletion or anergy of T cells. In contrast, crosspresentation in the context of viral infection or immunopathology generates either potent protective or harmful immune responses, respectively. Among danger signals, type I interferons (IFN-α/β) have been shown to be critically involved in crosspriming and crosspresentation. Because pDCs (also called interferon-producing cells) are widely known as professional type I IFN producers, an ancillary function of this cell type in crosspriming and crosspresentation would be expected. However, the antigen-presenting capacity of pDCs has long been matter of debate. Human pDCs can expand alloreactive T cells or melan A-specific cytotoxic T lymphocytes (CTLs) in vitro. They can also polarize proliferating T cells toward T helper 1 (Th1), Th2, or regulatory T cells, depending on their activation state. Nevertheless, given the high precursor frequency of alloreactive or melan A-specific T cells, the ability of pDCs to elicit responses from rare, truly naive $CD4^+$ or $CD8^+$ T cells remains questionable.

The identification of murine pDCs provided the means to rigorously assess the capacity of these cells to present antigens in vitro and in vivo. In vitro data indicated that classical DCs present antigens more efficiently and elicit more potent T cell responses than do pDCs. These in vitro results suggested that pDCs, like B cells, predominantly induce memory and recall responses. However, in vivo experiments suggested otherwise: If a soluble antigen is targeted to the pDC receptor BST-2, murine pDCs can prime transgenic naive $CD4^+$ T cells in specific anatomical compartments, such as peripheral lymph nodes, although they fail to provoke a concomitant specific $CD8^+$ T cell response (Sapoznikov et al., 2007). Murine pDCs can also crosspresent soluble antigen to transgenic T cells in vitro upon CpG stimulation, a pathway that requires osteopontin-dependent IFN-α secretion (Shinohara et al., 2006). It should be noted, however, that the frequency of naive T cells with a given specificity in these experiments is unusually high because TCR transgenic T cells were used. In fact, pDCs pulsed with soluble antigens and activated by CpG fail to induce substantial expansion of specific CTLs from rare naive precursor T cells in vivo, unless antigen is targeted to a specific endocytic receptor, called Siglec H, that is expressed on pDCs (Zhang et al., 2006). Thus, murine pDCs can crosspresent, but only in certain experimental settings—targeted delivery of the antigen, high frequency of T cell precursors, or Toll-like receptor (TLR)-induced activation with CpG. Whether these conditions are frequently met in vivo is unclear.

There is no convincing evidence that human pDCs can crosspresent antigens. Schnurr et al. demonstrated that immune complexes of exogenous melanoma-derived antigens conjugated to immunoglobulins are efficiently presented to specific CTLs by primary myeloid DCs but not by pDCs, even when activated by TLR agonists (Schnurr et al., 2005). However, in this issue of *Immunity*, Hoeffel et al. (2007) provide experimental evidence that human pDCs can indeed crosspresent HIV-derived peptides conjugated to a lipopetide (a candidate vaccine for HIV infection), as well as HIV-infected cells undergoing apoptosis (Figure 1). During the crosspresentation process, antigen is taken up in an endosomal compartment marked by the endocytic receptor BDCA2 and afterwards localized in low-acidic vesicles. Surprisingly, in these experimental settings, pDCs crosspresent antigen as efficiently as do myeloid DCs. Crosspresentation is enhanced by stimulation with influenza virus, suggesting that it might be dependent, at least in part, on type I interferon.

Mechanistically, crosspresentation by pDCs requires proteasome activity, indicating that antigen is either passively delivered or actively transported into the cytosol for further processing. The molecular mechanisms that govern the crosspresentation pathways in pDCs, as well as the precise nature of the endosomal compartment in which crosspresentation occurs, await future studies. However, because pDCs have abundant endoplasmic reticulum (ER), it is tempting to speculate that ER-phagosome fusion might occur at high rate in pDCs, bringing together exogenous antigen, the MHC class I loading machinery and, potentially, proteins involved in the ER-retrotranslocation system, such as the AAA ATP-ase p97 (Ackerman et al., 2006). In addition, crosspresentation might be enhanced in pDCs after stimulation with TLR7 or TLR9 agonists and

viruses. The abundant secretion of type I interferon might activate NOX2 or other components of the nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase system, which produces reactive oxygen intermediates (ROIs). ROIs reduce endosomal pH, which in turn prevents complete degradation of the antigen and thereby enhances antigen crosspresentation (Savina et al., 2006). Although human pDCs do not express mannose receptor, a C type lectin that favors crosspresentation by enabling antigen uptake into specialized endosomes (Burgdorf et al., 2007), they might employ other mechanisms of receptor-mediated endocytosis to crosspresent antigens. This phenomenon could also explain the discrepancy between the crosspresentation of HIV peptide-lipopeptide conjugates and HIV-infected apoptotic cells observed in the present study, and the lack of crosspresentation of tumor antigens or tumor antigens delivered as immune complexes (Figure 1). It is likely that, depending on the receptor selectively engaged, the antigen reaches different compartments and undergoes complete degradation and exclusive loading onto MHC class II or partial degradation and further processing into the cytosol for loading into MHC class I molecules. Accordingly, myeloid DCs are more efficient at crosspresenting HIV-gag protein when it is targeted to human-DEC205 receptor than when it is targeted to a closely related receptor like DC-SIGN (Bozzacco et al., 2007).

Hoeffel et al. have returned pDCs to the center stage of antigen presentation. Clearly, it is essential to determine whether crosspresentation by human pDCs is a common event, especially during HIV infection, and whether such presentation leads to T cell stimulation or T cell anergy in vivo. Because HIV does not productively infect pDCs, but activates them through TLR7, crosspresentation by pDCs might be a very effective way to elicit anti-HIV T cell responses.

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Why Is There so Much CD45 on T Cells?

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The balance between kinases and phosphatases is crucial for regulating lymphocyte signaling. In this issue, McNeill et al. (2007) show that the transmembrane phosphatase CD45 has a role as both positive and negative regulator of T cell signaling.

The presence of CD45 molecules on hematopoietic cells has long been an enigma: Why so much, why so big, why so variable, why can we not find a ligand? One of the most abundant molecules on the lymphocyte surface, CD45 was identified a number of years ago as a transmembrane phosphatase (Hermiston et al., 2003). The large extracellular domain of CD45 is notable for its highly glycosylated and sialylated state, which varies depending on the inclusion or exclusion of alternatively spliced exons 4, 5, and 6. The resulting isoforms are specific not only to hematopoietic cell type, but also to the stage of differentiation and activation of the cell. Not surprisingly, therefore, it was suggested that these alternative isoforms might interact with unique ligands; however, convincing identification of ligands specific for any of the isoforms has so far defied a seemingly endless supply of research dollars and several lifespans of graduate students.

The major intracellular targets of CD45 phosphatase activity are the Src-family kinases (SFK), which in T cells are predominantly the family members $p56^{Lck}$ (Lck) and $p59^{Fyn}$ (Fyn). Lck, in particular, is a primary initiator of signal transduction upon